THE DETERMINATION OF PARAQUAT (1,1'-DIMETHYL-4,4'-BIPYRIDYLIUM CATION) IN URINE

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SUMMARY

Three methods of paraquat analysis have been examined and a suitable procedure for clinical laboratory use is recommended. The method is rapid and can determine down to 0.01 *μg* paraquation/ml in a 250-ml aliquot of urine. A rapid spot test with visual sensitivity of 1–1.5 *μg* ion/ml is also described. Some urine levels following oral ingestion of paraquat are reported, but it was not possible to correlate the levels found with severity of poisoning.

INTRODUCTION

Since its introduction as a non-selective herbicide, the bipyridyl compound paraquat has gained worldwide popularity. The main reasons for this are the rapid onset of herbicidal action and, perhaps more important, since the compound is rapidly absorbed to soil particles the residues are not available to plant roots. It is marketed as granules containing 5% of active ingredient or as a liquid concentrate containing 20%, only the former being sold for home garden use. Both preparations are diluted with water before application.

On the basis of animal work paraquat was regarded as only moderately toxic. In man, however, it has gained a reputation as a so-called "hit-and-run" poison because after a single oral dose, and at a time when most of the chemical ingested has left the body, there is a development of hepatic, renal, myocardial and pneumonic changes, the last of which usually prove fatal. The toxic mechanism at the cellular level has not been completely elucidated and until more is known about the manner in which paraquat triggers off this characteristic proliferation reaction in the lung it is unlikely that effective or radical treatment can be instituted. Meanwhile, it seems expedient to encourage prompt elimination of the compound from the body by early and intensive diuresis. Before embarking on such a regime it is essential: (a) to ascertain quickly that paraquat is the chemical involved and also (b) that a significant quantity has been absorbed.

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This paper describes tests for these two purposes and reports some results of urine analysis following paraquat ingestion.

MATERIALS

Paraquat dichloride was a gift from Imperial Chemical Industries Ltd. Since it is hygroscopic it was dried for 5 h at 100° before use[1]. Dowex AG50W-X 8 (50–100 mesh) and Dowex AG50W-X 8 (100–200 mesh) were obtained from Micro Bio Laboratories, London. All other chemicals were of Analar grade and purchased from Hopkin & Williams, Chadwell Heath, Essex. The sodium dithionite was stored in a vacuum desiccator.

EXPERIMENTAL

Spot test evaluation

Urine samples were collected from six different people and paraquat solutions prepared in these urines at the following concentrations: 1.0, 2.5, 5.0, 7.5, 10.0 μg/ml ion. Ten ml aliquots of each solution were taken and 2 ml of 1% sodium dithionite in 1 *N* sodium hydroxide added. A visual inspection was immediately performed for the presence of the blue free radical. A narrow range of standards was also prepared to give a closer check near to the limit of detection. The evaluation was performed by five people who had little experience of the colour of the free radical. It was found that 1.0 μg/ml ion could be detected in a clear urine and 1.5 μg/ml ion in cloudy, coloured urine with very good reliability. Tompsett[2] has put his visual limit of detection at 20–50 μg/ml.

Quantitative urine studies

Three alternative urine preparations were investigated prior to ion-exchange chromatography:

Untreated—Urine without any pH adjustment was passed through the ion-exchange column.

Hydrolysis—A measured volume of urine was adjusted to 2 *N* with 18 *N* sulphuric acid and the sample boiled under reflux for 2 h. To aid gentle boiling and avoid any local overheating, glass beads were added to the flask. After cooling the condenser was washed down with water and the sample diluted until the acid strength was below 0.5 *N* before percolating through the ion-exchange column.

Trichloracetic acid treatment[3]—To the measured volume of urine, one-quarter volume of 25% TCA was added to give a final concentration of 5% TCA in the mixture. After centrifuging for 15 min, the supernatant was decanted and the retained precipitate resuspended in 50 ml of 5% TCA and centrifuged again for 5 min. The second supernatant was combined with the first and percolated through the ion-exchange column.

Ion-exchange chromatography

Ion-exchange columns were prepared by placing glass wool plugs in the bottom of 50-ml burettes and washing 3 g of Dowex AG50W-X 8 (50–100 mesh) on to the plug with distilled water. The prepared samples were passed through the resin at a flow rate not greater than 5 ml/min. The column was washed successively with 100 ml distilled water, 100 ml *N* hydrochloric acid, 50 ml distilled water, 100 ml 2.5% ammonium chloride and 100 ml distilled water allowing the latter to drain just to the top of the resin which was not allowed to become dry at any stage. The resin was finally eluted with saturated ammonium chloride solution at a rate not greater than 1 ml/min and the first 50 ml collected in a volumetric flask.

Colorimetric measurements

Standard solutions of paraquat dichloride were made up in saturated ammonium chloride solution to contain 0.25, 0.5, 0.75 and 1.0 µg/ml of paraquat ion. These solutions are stable although exposure to strong sunlight should be avoided.

A reducing solution containing 1% sodium dithionite in 1 *N* sodium hydroxide was prepared and used within 2 h.

Ten ml of each standard were pipetted into 19-ml glass-stoppered tubes and 10 ml of saturated ammonium chloride were pipetted into a similar tube to use as a reference solution. Two ml of the dithionite-reducing solution were added to each tube and the absorbance was measured at 396 nm in 4 cm glass cells. Since this free radical is only moderately stable, absorbance values must be obtained within 5 min of adding the reducing reagent. A reliable procedure was to add dithionite solution to the first standard and reference solution, stopper, mix gently by inversion and read immediately. The reducing agent was then added to the next standard and the determination repeated.

Samples were similarly treated by pipetting 10 ml of each eluate into a tube and adding 2 ml of the sodium dithionite solution as before. The absorbance of the samples was then measured at 392, 396, 400 and 401 nm. Occasionally, it was necessary to dilute the eluate with saturated ammonium chloride to obtain satisfactory readings.

Calculation

A calibration graph of absorbance against concentration was prepared and this obeyed the Beer–Lambert Law (Fig. 1).

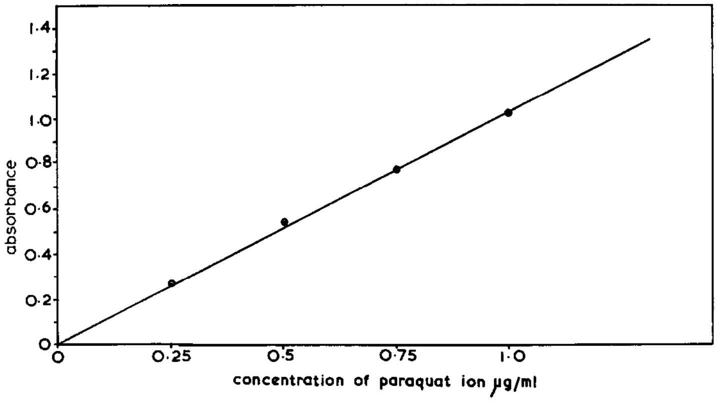

Fig. 1. Calibration graph relating absorbance to paraquat ion concentration.

The absorbance at 396 nm (E_{396}) of the sample solution is corrected for linear background absorption by means of the following equations[1]:

$$E'_{396} = 2.91E_{396} - 1.61E_{392} - 1.28E_{401} \qquad \text{(A)}$$
$$E''_{396} = 1.68(2E_{396} - E_{392} - E_{400}) \qquad \text{(B)}$$

The mean value of the two corrected absorbances is then related to the calibration graph and hence the concentration of paraquat ion in the eluate is obtained.

$$\text{Paraquat ion concentration in sample} = \frac{a \times v}{V} \times \frac{100}{\beta}$$

where a = paraquat ion concentration in eluate
v = volume of eluate
V = volume of urine analysed
β = % recovery

The factor β is determined by doing standard additions and recoveries in the range encountered.

Sensitivity

The sensitivity of the method is 0.01 µg ion/ml in a 250-ml aliquot of urine. We chose to measure the absorbance at 396 nm, rather than the less sensitive peak at 600 nm preferred by some authors[3]. The molar extinction coefficients of the paraquat ion in ammonium chloride at 396 nm and 600 nm are 38000 and 9000 respectively—a difference which possibly may, in part, account for the varied views about the time paraquat can be detected in the urine after ingestion.

RESULTS AND DISCUSSION

Paraquat, being a highly polar compound, is very soluble in water. The most convenient way of concentrating traces present in aqueous solutions and separating them from interfering compounds is by means of a cation exchange resin. Two resins were known to be suitable: Zeo-Karb 225 (8% DVB) 50–100 mesh and Dowex AG50W-X 8 (100–200 mesh). We also tried Dowex AG50W-X 8 (50–100 mesh). Our results regarding absorption and elution from the first two resins were in agreement with Calderbank and Yuen[1]. However, we preferred to use Dowex AG50-X 8 (50–100 mesh), which also gave quantitative recoveries, but required no resin preparation before use. The larger mesh size did not suffer from slow flow rates as did the smaller mesh Dowex resin tried by previous authors.

Recovery experiments

Recovery experiments were performed by adding paraquat ion to urine at the levels 0.04, 0.1, 0.2 and 1.0 µg/ml and comparing each of three procedures. At the higher levels only 50 ml of urine was analysed, but for lower levels it was necessary to take a larger sample volume. After extraction and column concentration, paraquat was determined by reduction to a moderately stable free radical as described above. The results are shown in Table I.

A further check was made by analysing samples from patients who had ingested

TABLE I

RECOVERIES OF PARAQUAT ADDED TO URINE IN THE RANGE 0.04–1.0 μg/ml BY THREE DIFFERENT PRETREATMENTS

Treatment	*Average % recovery*
No treatment before column chromatography	86.0
Acid hydrolysis	85.0
Trichloracetic acid precipitation	77.5

paraquat, both by direct ion exchange chromatography and after acid hydrolysis. There was no difference in the results, and so it can be concluded that after passage through the body, no paraquat is contained in urine that is unavailable to the resin without preliminary hydrolysis. Direct percolation of the urine samples through an ion-exchange column with no pretreatment step is, therefore, the most rapid and convenient method for clinical laboratory use.

Chromatography

Some workers have used chromatographic methods of detecting paraquat[4]. These will be fraught with difficulty because the highly polar nature of the compound makes its extraction and concentration by organic solvents impossible and any results obtained by these methods must be viewed with suspicion.

Patient studies

Apart from single determinations, we have done long term excretion studies by this method on five patients following paraquat ingestion. The medical findings in

TABLE II

ANALYSIS OF 24-h URINE COLLECTIONS FROM TWO PATIENTS FOR PARAQUAT ION

D.B			*M.S.*		
Sample number	*Volume (ml)*	*Total μg PQ^{2+} ion voided*	*Sample number*	*Volume (ml)*	*Total μg PQ^{2+} ion voided*
Initial	475	5700	Initial	7400	5000
—	—	—	1	7000	2330
2	2500	669	2	2900	840
3	5935	1089	3	1240	300
4	5730	989	4	1760	380
5	9000	954	5	2190	320
6	6850	973	6	2270	660
7	6920	1509	7	2560	530
8	7500	1920	8	2030	740
9	8200	1476	9	1700	550
10	10600	1696	10	1750	280
11	10000	1600	11	1450	200
12	3700	1439	12	1330	170
13	1850	805	13	1400	180
14	3870	1072	14	1230	70
15	3000	642	15	1100	50
16	2700	594	16	850	70
17	3000	396	17	750	60
18	2900	168	18	950	50
19	2900	197	19	490	10
			20	146	20
			21	460	20
			22	1080	20
			23	1295	20

TABLE III

ANALYSIS OF URINE COLLECTIONS FROM THREE PATIENTS FOR PARAQUAT ION

Days after ingestion	*D.F.*		*M.H.*		*S.H.*	
	Urine volume (ml)	*Total μg PQ^{2+} ion voided*	*Urine volume (ml)*	*Total μg PQ^{2+} ion voided*	*Urine volume (ml)*	*Total μg PQ^{2+} ion voided*
1	NC	—	540	2160	NC	—
2	NC	—	NC	—	NC	—
3	NC	—	NC	—	NC	—
4	NC	—	150	390	NC	—
5	NC	—	NC	—	NC	—
6	NC	—	NC	—	230	21
7	NC	—	790	103	NC	—
8	NC	—	NC	—	200	19
9	NC	—	1100	550	180	18
10	3846	1846	NC	—	NC	—
11	4340	1754	NC	—	NC	—
12	3490	771	NC	—	91	3
13	NC	—	NC	—	175	3

NC = No collection.

two of these cases will be reported later and Table II shows a comparison between the analytical figures in each case. The remaining three cases were not followed in the same detail, Table III, because many of the samples were not collected.

It can be seen that the work of Matthew *et al.*[5] which gives evidence for the long term excretion of a small proportion of the paraquat is wholly substantiated. Earlier work with rats and cows[3,6], had indicated that in these species the total ingestion of paraquat could be excreted in 48 h. Table II shows that excretion of paraquat in these subjects is still occurring three weeks after oral ingestion. There is some evidence, in one of these results, to suggest that a relationship exists between the total weight of paraquat excreted and the volume of urine voided. This effect was prominent in the case of D.B. once the kidneys had recovered from the initial damage to them as indicated by a high blood urea. A similar dependence of paraquat clearance upon urine flow was reported by Kerr *et al.*[2], who postulated that glomerular filtration rate does not govern the renal excretion of paraquat. On this basis, Swann recommends an immediate induction of diuresis following ingestion[7] and hence the need for urgency on the part of the laboratory to confirm that paraquat has been ingested.

Spot test

Several cases of paraquat ingestion have been reported in the medical press (refs. 2, 5, 8–10 and) it is obvious from cases reported by other workers and ourselves in this present paper, that it is difficult to relate severity of poisoning and eventual outcome to urine levels alone. Swann has stated that patients with urinary paraquat concentrations still above 0.2 mg/100 ml (i.e. 2 μg/ml) three days after ingestion should be hospitalised for the ensuing ten days. We are inclined to believe that even lower levels should be regarded as of sinister portent. Any patient who gives a positive reaction to our "spot test", which is sensitive to 1.0 μg/ml, has in our view absorbed such a quantity of paraquat that dangerous reactions may well be exhibited. We would, therefore, advise that any patient in this category be carefully observed for at least ten days, paying careful attention to renal, hepatic, myocardial and above all respiratory function.

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